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The National Environmental Specimen Bank

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On August 19 and 20th, 1976, the National Bureau of Standards and the U.S. Environmental Protection Agency co-sponsored a Workshop to review technical developments and to make recommendations on implementation of the National Environmental Specimen Bank. The Workshop consisted of a review session where past considerations were discussed; a technical session where recent analytical research relevant to the sample bank was abstracted and discussed; and a planning session where planning and design of a prototype banking system was outlined.

This report is a summary of the presentations, discussion, and conclusions of the Workshop attendees. The attendees represented a wide cross section of interested Federal and Non-Federal research groups as well as International representation including the International Tissue Banking Program (Sponsored by the World Health Organization, The Commission of European Communities and the U.S. Environmental Protection Agency) and the Federal Republic of Germany.

The workshop concluded that with the ever increasing influx of new man-made substances into our ecosystem, that a formalized, systematic approach is needed to assess the environmental impact of these substances on a national as well as an international level. The technology to initiate a pilot banking program is presently available and was formulated into a five-year pilot bank program. This program will be evaluated at each stage of development.

This report was submitted in partial fulfillment of EPA Interagency Agreement IAG-D4-0568 by the National Bureau of Standards under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers the period August 19 and 20, 1976, and the work was completed as of January 31, 1977.

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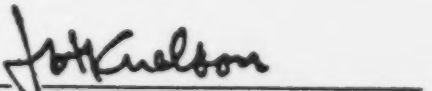
FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

As part of the Health Effects Research Laboratory's efforts to provide a coordinated environmental health research program, the U.S. Environmental Protection Agency and the National Bureau of Standards co-sponsored a Workshop to review current technical developments and to make recommendations affecting the proposed National Environmental Specimen Bank (NESB). The NESB is part of an International effort to monitor the environment for hazardous substances. The advantages of such a program will permit the Agency to assess the effectiveness of its present environmental control techniques by monitoring pollutant trends, as well as establishing environmental baseline levels of new pollutants or pollutants of current concern not previously investigated.



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Key Words: Analytical; National Environmental Specimen Bank; prototype; research; sample bank.

TABLE OF CONTENTS

		Page
Foreword		iii
Abstract		iv
Figures		v
Acknowledgment		vi
1.	Introduction	1
2.	Background and History	3
3.	Results of Current Research Programs	7
4.	Discussions and Recommendations	10
5.	Conclusions	14
Bibliography		18
Appendices		19
A.	The Cleaning, Analysis and Selection of Containers for Trace Element Samples.....	19
B.	Evaluation by Activation Analysis of Elemental Retention in Biological Samples after Low Temperature Ashing	33
C.	Determination of Chromium in Biological Matrices by Neutron Activation: Application to Standard Reference Materials.....	43

FIGURES

Number		Page
1	National Environmental Specimen Bank (NESB) Concepts.....	15
2	Pilot Bank samples.....	16
3	NESB Pilot Bank.....	17

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DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

SECTION 1

INTRODUCTION

On August 19 and 20th, the National Bureau of Standards (NBS) and the Environmental Protection Agency (EPA) co-sponsored a Workshop to review current technical developments and to make recommendations affecting the proposed National Environmental Specimen Bank (NESB). The attendees represented a wide cross section of interested Federal and non-Federal research groups as well as representatives from The Federal Republic of Germany and the International Tissue Banking Program, sponsored by the World Health Organization (WHO), U.S. E.P.A., and Commission of European Communities (CEC).

The Workshop was conducted as an essential part of the program planning, preliminary to the establishment of the National Environmental Specimen Bank. There has been a concerted effort on the part of EPA and NBS to initiate the NESB system. This system will provide a dual output useful to many environmental monitoring and assessment programs; that of credible real time monitoring data for an environmental early warning system and that of providing well preserved and documented environmental samples for future retrospective analysis.

The Workshop was divided into three half-day sessions. The first session was a review of the rationale and objectives of the NESB system. The second session was a review of scientific research pertinent to NESB objectives. The final day was devoted to a planning and design of a prototype system for the banking and analysis of environmental samples. During this planning session, the following considerations were specifically addressed:

1. A review of the issues that created the need for the National Environmental Specimen Bank with specific emphasis on current environmental problems which would be aided by the existence of a sample banking system.

2. The development of specific objectives, identifying the functions that the bank would perform, and how it would meet the needs as specified in the rationale statement.

3. Identify the types of specimens that would be stored in the bank, including demographic and technological data, based upon existing and anticipated future needs and objectives.

4. Identify and evaluate the potential users of the bank and set forth guidelines to govern those who could use the bank and what could be drawn from it.

5. Determine if the technology is presently available to initiate a pilot program.

With the successful completion of these tasks, a plan was generated for the establishment of a pilot bank program. This pilot bank will utilize scaled-up laboratory procedures established at NBS as part of the NBS/EPA cooperative effort to provide standardized sampling, storage and analytical protocols for environmental samples, and identify additional areas of research and development that would be needed before the full National Environmental Specimen Bank is formalized.

SECTION 2

BACKGROUND AND HISTORY

The storage of human tissues and other environmental samples for analytical measurements is a natural outgrowth of the EPA environmental monitoring system. The need for environmental monitoring is well established and is currently a major EPA responsibility. A comprehensive monitoring system is an integral part of the EPA mandate to assess the total environmental impact of pollutants that may lead to deleterious effects on human health.

The health research objectives of EPA can be stated as:

1. To minimize adverse human effects
   - Prevent exposure to harmful new agents
   - Reduce exposure to existing agents
   - Predict relative effects of control options

2. To quantitate the benefits of environmental control.

3. To optimize the environment for man's health and well-being.

A preliminary program using human tissue samples as monitors for trace element pollutant burdens was initiated in the EPA human pollutant burden studies program.

From the very onset of the human pollutant burden studies, short-term tissue banking became an integral part of this program. Human tissues were viewed as environmental dose integrators of multiple proportions. The integration of a pollutant level with time was used to estimate pollutant trends. Analyses of these pollutant trends served as a resource for standards setting, inputs for cost-benefit assessments of pollution abatement measures, and guides for determining research priorities.

The problem orientated approach of the early pollutant burden studies did not fully utilize the capabilities of a tissue banking program. At that time, a tissue or group of tissues was collected to test a specific hypothesis. These tissues were stored until they could be analyzed, and were then discarded. As the pollutant burden program expanded, the need for a fully developed tissue banking system became apparent.

This banking system was envisioned as having a dual function. First, representative portions of samples included in the bank would be analyzed at the time of introduction to provide real time monitoring and evaluation of pollutant trends. Evaluation of these trends would serve as early warning sentinels so that proper control measures could be taken to halt rising human body burdens before irreversible damage could occur. Second, a specimen bank would enable the analyst to use tomorrow's more sensitive and specific methods of chemical analysis on today's samples. The improved measurement methodology would enable health scientists to determine accurate levels for substances that would be either undetectable or poorly analyzed by today's less sensitive methodology. The existence of a specimen bank would provide the opportunity to determine what the body burden of newly recognized toxic substances was in the past and to determine if their levels had changed with time.

In EPA's continuing effort to establish a National Environmental Specimen Bank, a two day working session was held in February 1973 at EPA's National Environmental Research Center, Research Triangle Park, North Carolina to discuss and propose plans for the establishment of a National Environmental Specimen Bank System.

The broad objectives of this working session were:

1. Establish current trends in human pollutant burdens (short-term banking).

2. Creating a specimen bank that would provide retrospective analytical capability (long-term banking).

One major recommendation from the working session was that a sample banking system should be established at the national level that would cross agency lines and provide human tissues representative of that period in time from which the sample was taken. The proper storage of these tissues would permit retrospective analysis using improved methodologies that were likely to be available.

In 1972, the National Academy of Sciences/National Research Council (NAS/NRC) addressed tissue banking when they stressed the lack of coordination in the numerous programs by components of Federal and State governments, private industry and academic institutions to collect, store and analyze specimens of environmental interest.

In an effort to upgrade the availability and long-term protection of environmental samples and to make the information gathered with each collection readily available, the Subcommittee on the Geochemical Environment in relation to Health and Disease of the National Committee for Geochemistry, National Academy of Sciences/National Research Council at their Asilomar Workshop in California (1972) recommended that a group of specialists be convened to study this problem at its Capon Springs Workshop in May 1973.

The Capon Springs Workshop concluded that the U.S. Government should establish, on a permanent basis, a National Environmental Specimen Bank. Initial coordinating and funding of the multi agency system should be considered by the National Science Foundation (NSF). The Environmental Protection Agency should be considered as the most logical organization to establish the system.

Four tasks were proposed to begin development of the NESB.

Task I. Conduct an inventory and assess the value of existing specimen collections as potential candidates for participation in the NESB.

Task II. Establish a steering committee composed of representatives from a variety of concerned groups that are providing funds, participating in specimen collection, and operating monitoring programs. This committee would be responsible for:

1. Developing the organizational and managerial structure of the NESB.

2. Identify the types of specimens and information to be stored in the Banking System.

3. Develop interim protocols for sampling, sample handling, and storage of specimens to be included in the bank.

4. Plan for a data handling, storage, and retrieval system.

Task III. Identify research needs as determined during the implementation of the NESB. Areas already identified are:

1. Sampling strategies

2. Sample processing and storage procedures

3. Measurement strategy

Task IV. Conduct meetings at the national and international level, of user and research groups, to exchange current information that would be relevant to the NESB.

During this time, the rapid growth of EPA's human pollutant burden program dictated the need for a sophisticated system of standardized protocols for sample collection, preparation, storage and analysis.

In December, 1973, this need was addressed by the EPA and the NSF in a meeting to formulate plans for the development of a National Environmental Specimen Bank. At this meeting, a four point proposal was generated, with joint funding being provided by NSF, EPA and NBS. This proposal was designed to set the ground work for establishing a specimen bank; a bank that would meet the requirements of both Federal and State regulatory agencies and that of the academic community.

The four point proposal contained the following elements:

1. A survey of existing specimen collections

2. An evaluation of existing specimen collections

3. Research and development of sampling, storage, and analysis protocols

4. The development of a planning document for the organization and management of the NESB

The specimen collection survey, conducted nationally, was used to generate a broad data base on various aspects of specimen banking. This survey was conducted by the Oak Ridge National Laboratory.

NBS then had the responsibility of critically evaluating the survey results to determine their utility and applicability to the National Environmental Specimen Bank program.

In general, the survey evaluation revealed that the collections, sampled and stored for purposes other than retrospective analysis, provided little information that was applicable to the analytical nature of the specimen bank program. Their use, however, was primarily in the area of taxonomy (1).

Concurrent with the survey, NBS began and is continuing research to generate state-of-the-art methodology for sample collection, preparation, storage and analysis. This task is envisioned as a continuing function for the life of the bank. This approach would allow for a continued updating of methodology to meet the needs of the bank.

Finally, the last task was the formulation of a plan for the development and operation of the bank. The clarification of this plan with respect to several key issues was to be the final result of the present Workshop. Relevant scientific issues which were discussed were:

1. Review issues and need for the bank

2. Development of specific bank objectives

3. Identify and specify sample types

4. Specimen collection, preparation and storage requirements

5. Evaluate and formulate methods of analysis

SECTION 3

RESULTS OF CURRENT RESEARCH PROGRAMS

Since January 1975, the Analytical Chemistry Division of the National Bureau of Standards has conducted a continuing research program to improve methodologies for the collection, storage, and analysis of NESB samples.

The program currently underway is pursuing three avenues of investigation. The first is a complete survey of available literature concerning problems of sampling, transporting, and storage of biological and environmental samples for analytical purposes. The second is an active research program to improve methodologies for sampling, sample handling, and storage of the above matrices. The third area concerns the evaluation and improvement of analytical techniques to be used for the analysis of the trace constituents of interest. The latter two portions of the NBS research program are currently directed primarily towards trace elements, but future research will be directed toward other substances of interest, such as trace organic species.

Literature Survey

The survey of recent literature entailed the use of both manual searching and computer assisted bibliographical retrieval services. These included Medline, Chemcon, Biosis, Cain, Defense Documentation Center, and others. The specific components of interest that were researched included trace elements, pesticides, other trace organics, radionuclides, and microbiological species. This survey, containing over 200 references, represents a single base of scientific data for the development of guidelines for sampling techniques, container cleaning methods and storage techniques (2). In general, the information found in the literature survey was limited, often contradictory, and usually pointed out problems rather than solutions. Several useful points of information that were documented include references on trace element contamination of biological tissues due to stainless steel sampling implements, and the substantial problems associated with currently accepted water sampling and storage techniques.

Sampling and Storage

A major portion of the current NBS research program has been the experimental evaluation of contamination and losses of the trace constituents of interest during sampling, sample handling, and long-term storage. One of the initial projects in this program was the evaluation of twelve

polymeric materials for their trace element content, and for the possibility of removing these trace elements when contacted by liquid samples. This study was made using three complimentary trace analytical techniques, neutron activation analysis, atomic absorption, and spark source mass spectrometry. The utilization of a multidisciplinary analyical approach gave an almost complete coverage of trace elements of interest.

The results of this study indicated that many materials were grossly contaminated by trace elements from plasticizers, formulators, and other process materials. However, conventional polyethylene and Teflon were found to be reasonably clean and it was generally found that less than 10 percent of the bulk trace element content could be leached out, even with conditions as severe as a 2-hour hot leach with 6N acid. The complete details of this study are included in Appendix A.

A second, and equally important part of the current research program has been a study and evaluation of long-term storage techniques which would be adequate for tissue and other biologically active samples. The effects of microbiological action on trace constituent concentrations and distributions are well documented. However, the mechanism for complete long-term elimination of that micro-biological activity is not well documented. Freezing has long been applied as a technique for analytical storage, however no study has yet been performed to document the reliability of this method of storage for more than a short period of time.

More recent studies into lyophilization have demonstrated minimal losses and/or contamination of trace elements during the sample processing. The NBS has now documented the viability of the freeze-drying technique to stabilize trace element composition. Standard Reference Material Bovine Liver (SRM 1577) has been shown to be unchanged for more than five years. The bulk material for this SRM was freeze-dried, ground, blended, and bottled. This material was analyzed and certified for trace element composition in 1972. To the present time, no documented evidence of trace element loss or alteration has occurred. The results of a complete study of losses on freeze-drying of liquid samples were published in Analytical Chemistry (3).

Finally, the technique of low temperature ashing (LTA) has been evaluated for long-term storage and found to have many advantages. A recent study at NBS investigated the loss of trace elements during plasma ashing using both radioactive tracers, and activation analysis of samples before and after ashing. The results obtained indicate that over thirty (30) trace elements are retained quantitatively during LTA. Five elements, mercury, osmium, and the halogens (chlorine, bromine, iodine), are not quantitatively retained. It was also determined from the above studies that contamination of the sample was not a measurable problem during ashing. An added advantage to the LTA technique is that resultant samples are easily composited and homogenized. The complete results of this research are detailed in Appendix B.

Trace Element Analytical Techniques

The third portion of the current research program has been to evaluate the effectiveness, and improve where necessary, the major analytical techniques for environmental samples. The elements specified to be of primary interest were mercury, lead, arsenic, selenium, nickel, vanadium, copper, manganese, beryllium, chromium, platinum, and palladium.

Since space does not permit a complete detailing of efforts in this area, two examples will be selected for brief discussion.

First, a complete study of the determination of chromium by neutron activation was undertaken. As has been reported recently, there is increasing analytical evidence which suggests the presence of a volatile organometallic chromium species in some types of biological samples. Recent work on the certification analysis of a candidate NBS SRM of brewer's yeast has indicated that neutron activation with radiochemical separation is able to determine reproducibly both the stable and the volatile species of chromium, and excellent agreement was obtained with other analytical results using closed system desolution. A complete description of this work is given in Appendix C.

The second example is the work at NBS on the modification of published procedures for the cold vapor atomic absorption determination of Mercury. Results from the determination of mercury in biological and environmental samples exhibited a disturbing variability with many results having a significant errors. A complete investigation of analytical errors, including losses during sample dissolution resulted in a detailed analytical procedure which now yields consistently reliable results down to the 50 parts per billion (ng/g) concentration level (4).

SECTION 4

DISCUSSIONS AND RECOMMENDATIONS

The second day of the Tissue Bank Workshop was devoted to a review of scientific issues and the formulation of recommendations specific to the implementation of the NESB. On review of the issues that created the need for the NESB, there was unanimous agreement that not only was the concept of sample banking still of vital importance to the assessment of low-level environmental contamination but that many of the original issues which mandated the implementation of the NESB were heightened due to recent environmental pollutant episodes. The Kepone episode in the James River and the Chesapeake Bay was pointed to as a prime example where existing specimens of documented validity would have been extremely useful to assess the change in the environment of that pollutant. There were not and are no samples of aquatic life or shell fish available from the Chesapeake Bay or James River which can be used for determination of Kepone levels before the start-up of the Kepone production in that area. Limited samples were available from the Virginia Institute of Marine Sciences dating back 3-4 years. These samples have proven invaluable in establishing the extent to which Kepone has affected the marine life. Had earlier samples been available from a banking system, a far better assessment of Kepone baseline levels prior to the dumping episodes would have been available to environmental officials. With the large increase of man-made chemicals now being put into our environment, it was concluded that issues such as the Kepone insult in Virginia are surely to be on the increase rather than to remain as an isolated situation.

A second possible function of the NESB system was raised and found by the participants to be of vital importance. It was noted that an NESB system should also include a real-time monitoring function. This function would provide trend analysis data for the real time estimate of our environmental quality in addition to assessing the effectiveness of our current pollutant control programs. This could logically be done by subsetting selected samples that were planned for inclusion in the bank and conducting real-time analyses on them. This would create an initial environmental monitoring program with vastly improved state-of-the-art analytical methodology. The storage of data produced by this system would also provide a mechanism for evaluating analytical methods with time. The combination of time dependent data with the availability of similar samples would allow a far superior method of quality control.

The single most important point to come from the Workshop was that the NESB can serve many important functions, not just that of long-term retrospective analysis. The results of sample banking would surely impact on

monitoring and health affects research of the EPA, and would be of great assistance to ongoing programs within the Department of Agriculture, Food and Drug Administration, and other agencies (Figure 1).

A set of specific objectives for the sample bank were identified during the second day's discussions. Those objectives are summarized as follows:

1. The collection, preservation, and storage of selected environmental samples using methodologies that had been documented to minimize or eliminate alteration of trace constituents.

2. The real-time analysis of selected trace constituents using methods of documented validity to obtain monitoring trend data.

3. Research in analytical methodology utilizing both the accumulated long-term data base and samples that have been stored in the NESB. This research will lead to a self-improving set of monitoring data.

4. The periodic review of the operation of the banking system relative to its valid input of samples and output of analytical data.

With the realization that the NESB is a viable concept, both by need and availability of technology, it was proposed that the NESB be initiated in the form of a five-year pilot program for banking environmental samples. This proposal was founded on the realization that a system such as NESB could quickly become overwhelmed with samples. This would invariably lead to a breakdown of the physical storage mechanisms, but more importantly, would likely lead to short cuts and compromises in operating procedures and analytical methodologies used for this real time monitoring phase of the system.

This position of moderate growth with careful monitoring of the program was unanimously recommended by the attendees of the Workshop. It was further recommended that the pilot program be initiated as soon as was feasible. During this time, limited numbers of samples would be collected, analyzed, and stored in a central facility. Problems encountered in the collection, transportation, analysis, and storage would be carefully monitored. Any difficiencies found in the system would be reexamined and new research would be initiated as necessary. With complete accord on the pilot scale concept, exact guidelines for this system were discussed and agreed upon.

The identification of specific sample types which should be included in the pilot sample bank during initial start-up of the system was discussed. The major focus of attention was on the absolute requirement to minimize both the number of samples and sample types in the pilot program so that the banking system did not become overwhelmed with either samples or analyses during its first years of operation. Unreasonably large numbers of avoidable errors would destroy its credibility before it was even in full operation.

Thus, all participants recommended the inclusion of a modest sample set per year for the first five years of operation with the focus on validating credible storage and analytical results.

A reasonable figure of approximately two thousand samples a year, split into four matrix types, was recommended (Figure 2). Also, it was recommended that the samples be obtained from no more than two different locations in the beginning phases, to minimize problems with logistics, sample collection, and transport.

Nine different sample types representing all major phases of environmental samples were considered for inclusion in the bank. These included air particulates, sediment, water, botanical, biological, and human samples. It was recommended that samples which represented environmental accumulators or integrators be emphasized as initial candidates, both due to the ease of analytical manipulation of those samples and because they represented time integrators of major pollutants present in our environment.

The first sample that was recommended for inclusion unanimously was a soft tissue sample that had an accumulator function in the human body, most likely liver or kidney. The second and equally important sample type was an accumulator of aquatic origin. Much discussion centered around the exact nature of the accumulator and suggestions ranged from plankton to shark tissue. Agreement was reached that a shellfish bivalve such as oyster which passes large quantities of water through its system every day and which tended to mirror increased concentrations of many toxic pollutants was a good choice. The third sample type was a food material representing a major input into the human diet. Consensus was unanimous that a food grain or composite of grains was the best choice. The fourth sample type was a collector of atmospheric or airborne pollutant materials. It was recommended that material such as lichen or moss was a good indicator of long-term trends in atmospheric pollutants and should be included in the initial bank. These four sample types were chosen for their diversity and their utility to environmental monitoring programs. However, a second and even more important consideration was that there is now enough scientific evidence to be reasonably assured that the storage and analysis of the trace element components in these materials could now be carried out with integrity.

One of the last items for consideration at the Workshop was the evaluation and formulation of viable analytical methods for the real-time and retrospective analysis of samples from the NESB system. Part of the NBS research effort has been to publish a compilation of analytical methods currently being used for SRM certification analysis. These methods will be available in the near future and were discussed at the Workshop. As the pilot bank program expands, additional analytical laboratories will become involved in the program. It was the concensus of opinion that rather than have a single specified analytical method be used on samples from the NESB, a better approach would be to rely on the judgment of high quality analytical laboratories to use methodologies that they had demonstrated as viable in their own laboratories. It is widely recognized that many analytical methods are capable of producing excellent results in the hands of a

talented analyst. However, in the improper hands, standard methodologies do not necessarily generate credible results. To the contrary, they usually generate a false sense of security which often leads to monumental mistakes. It was thus recommended by members of the Workshop that a compilation of recommended or evaluated methods be included in the NESB system, but these be included only for the information or aid to the analytical laboratories actually doing the work. The final decision on methodology would be left up to the capable laboratories or scientists working on samples from the bank. The laboratory capabilities would be under a strict quality assurance program with blind analyses of representative materials as a foundation.

As a summary to the recommendations and conclusions of the Workshop, a schematic drawing of the overall NESB system with its functions and responsibilities was put together in a composite form. This schematic represents most functions and outputs to be derived from the NESB system and is given in Figure 3. It was concluded by the members of the Workshop that a system such as this would give reliable data for the evaluation of trends in trace element and trace organic constituents of environmental and human nutritional importance over a long period of time.

SECTION 5

CONCLUSIONS

There is a real need for the evaluation of sampling, sample handling, and long-term storage methodology for the establishment of an effective environmental specimen banking system. This formalized, systematic approach to defining our current environmental hazards has never before been attempted on a national scale. Studies are continuing in a randomized fashion and in many cases without the proper validation of specimen banking procedures. It is this type of information, however, that is currently being used by the scientific community as well as State and Federal regulatory agencies to propose environmental quality standards and limits for control technology. If these types of monitoring programs are to continue, as they must to protect our environment as well as the health of our population, then the type of research program described here is required to establish and define the necessary basic scientific information required for such a specimen banking system. The NESB, when operational, will provide future generations with an important resource for evaluating their current environmental influences.

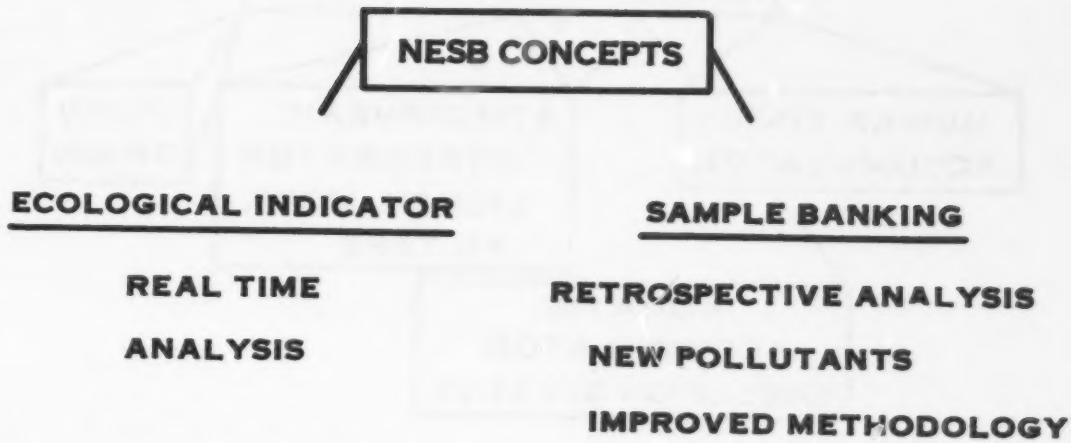

FIGURE 1. National Environmental Specimen Bank (NESB) Concepts.

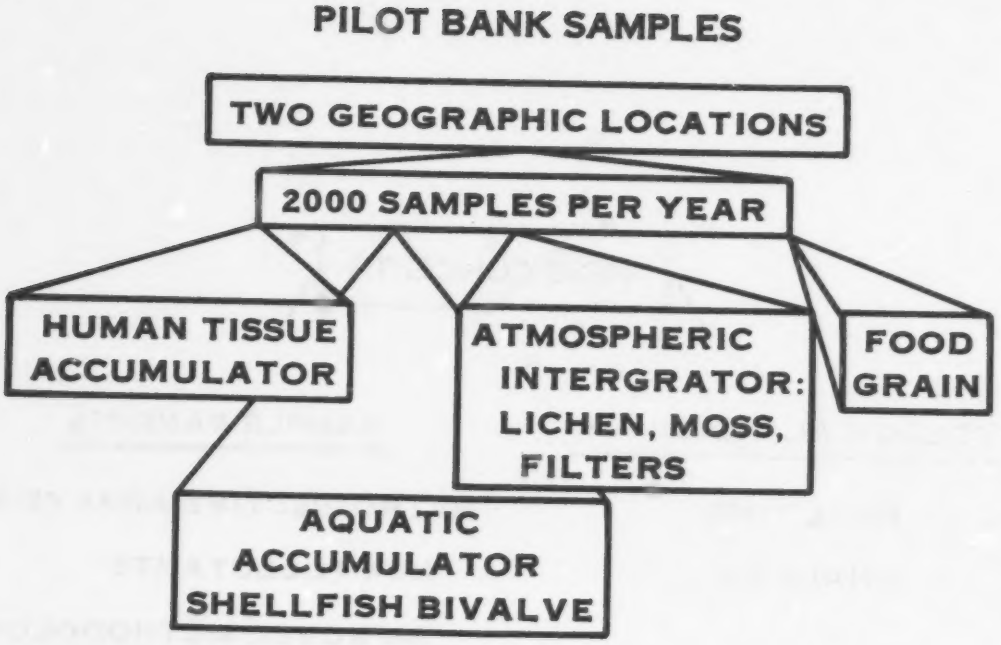

FIGURE 2. Pilot Bank Samples.

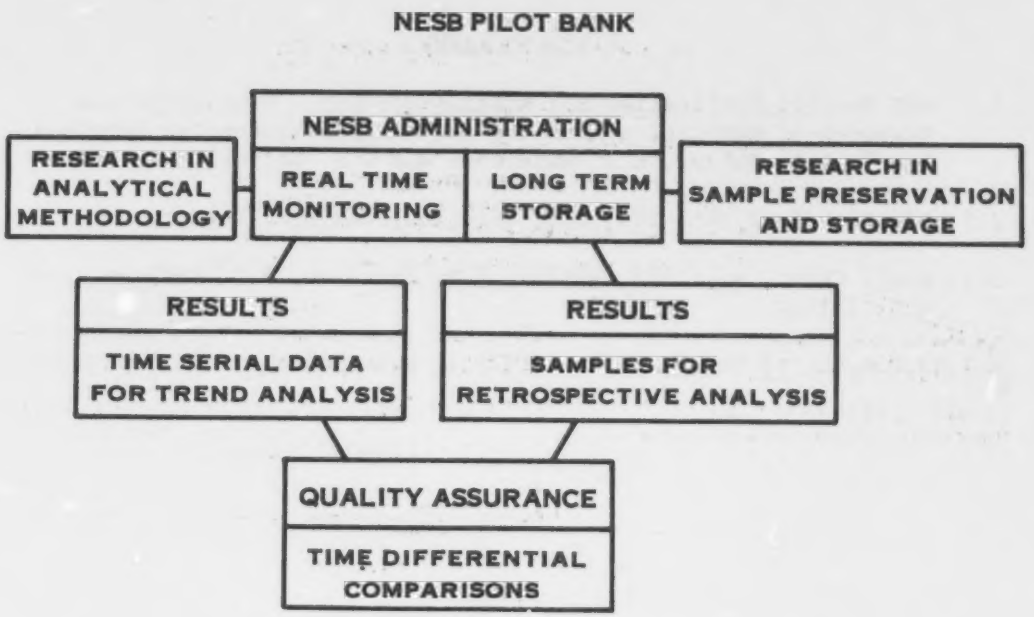

FIGURE 3. NESB Pilot Bank.

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APPENDIX A

THE CLEANING, ANALYSIS AND SELECTION OF CONTAINERS FOR TRACE ELEMENT SAMPLES

by

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ABSTRACT

Since the sample container represents one of the earliest and potentially one of the largest sources of sample contamination, much of the analytical accuracy will depend upon the choice of container materials and the method of cleaning containers. Twelve different plastics have been examined by gravimetry, isotope dilution mass spectrometry, and neutron activation analysis. The present study examines the impurities present in the various plastics as well as the actual level of impurities leached from the plastics.

Contemporary problems in analytical chemistry have forced the analyst to lower the threshold for determining trace elements to the parts per billion level (ng/g) or lower. More is involved, however, than simply lowering the detection limit for a method. Blanks, sampling, sample handling, and storage conditions often become dominant factors in a trace elemental analysis scheme. Maienthal and Becker (1) have recently reviewed the literature on sampling, sample handling, and storage. Problems are very severe for water and other liquid handling and storage. Generally, the usage of moderately concentrated mineral acid is considered the best way to prevent adsorption of trace metals from water by the container walls. While the best rule will continue to be to analyze a sample as soon as possible after sampling, the influence of the sample container on the analysis blank cannot be overlooked.

At these low levels (ng/g or less) the analytical blank in all of its manifestations becomes a dominant factor in the accurate determination of trace constituents in a sample. Patterson and Settle (2), for example, have reported that the majority of published data for lead in plants, animal tissue, and water is in error due to gross levels of contamination or failure to control the analytical blank. Murphy (3) has reviewed the role of the analytical blank in accurate trace analysis and defined its sources and methods of control. Often, as contamination is eliminated and other sources of the analysis blank are brought under control, the true concentration of a trace element is found to be even lower than previously determined.

Considering only the sample storage container, two primary methods of blank (contamination) control are available. These complementary techniques are the proper selection of container materials and the proper choice of a method of cleaning the container. Robertson (4) provided a good introduction to the subject by analyzing a number of reagents, construction materials, and container materials for various trace elements and then underscoring the implications for contamination of a sea water sample. The use of plastics and quartz has become common practice in most trace metal analysis laboratories.

Numerous authors have addressed the problem of containers and blanks, and suggestions for the proper way to clean laboratory ware and containers are almost as numerous.

Since so much of the literature concerning storage materials and cleaning procedures was contradictory (1), an effort was undertaken at the NBS to determine the best possible materials for sample storage containers. The immediate objective was to determine which container should be used for various applications such as Standard Reference Materials (SRM's) or the National Environmental Specimen Bank. Depending upon the application, considerations other than contamination levels must be anticipated (e.g., cost, commercial availability).

For liquid samples, an important consideration together with low contamination levels is a low rate of permeability to water vapor. Although such permeability data is available, it is difficult to apply to a commercially fabricated bottle. The wall thickness varies from plastic to plastic, from manufacturer to manufacturer as well as from bottle to bottle. This situation is also complicated by the relative efficiency of the closures provided. Thus, the experimental determination of the actual rate of loss of water from various containers was indicated.

Experimental

A number of plastic materials were studied by several methods. Among those materials examined were bottles constructed of conventional polyethylene (CPE), linear polyethylene (LPE), polypropylene (PP), polymethylpentane (PMP), polycarbonate (PC), polyvinylchloride (PVC) and several types of Teflon (TFE, FEP). In addition specimens of ethylenetetrafluoroethylene copolymer (Tefzel or ETFE), Teflon PFA, polystyrene (PS), and Teflon pipe sealing tape (TPT) were examined. The studies of these materials may be divided into three types of experiments: neutron activation analysis (NAA) of the bulk impurities within the plastics both before and after cleaning, isotope dilution mass spectrometric (IDMS) analysis of the impurities leached from a container during a cleaning process, and a gravimetric study of the loss rate of water from containers.

Gravimetric Studies. The loss of water from a plastic container by permeation is not trivial over a period of one year or more. Two containers each of CPE, PP, PVC, PMP, and PC were obtained from commercial sources. The containers were of a similar physical size. All of the containers were weighed on a high capacity analytical balance. Half of the containers were then filled with similar amounts of distilled water and re-weighed. The second container of each type was retained as a weighing tare to correct for the influence of changes in the barometric pressure, relative humidity and temperature and loss of plasticizer. The samples were re-weighed after 17 days and 66 days to establish a rate of loss of moisture.

IDMS Studies. Solutions contained in one liter or 500 mL size bottles of FEP, CPE, LPE, and PC were examined by spark source IDMS (multi-element) and by thermal emission IDMS (for lead only). The general principles and techniques

used have been given elsewhere (5,6). The bottles were rinsed with distilled water to remove any surface contamination and three sets of bottles were filled with a (1+1) mixture of pure HCl or pure HNO_3 and pure water (7). The bottles were allowed to stand full for one week, with the exception of the FEP bottle which was heated to 80 °C for one week.

Aliquots of the contents of one set of bottles leached by (1+1) HCl were spiked with ^{206}Pb. Aliquots from two other sets of bottles, leached by (1+1) HCl and (1+1) HNO_3, respectively, were spiked with a 19 element multi-spike (7) for the spark source mass spectrometer (SSMS). The spiked solutions were then evaporated to several drops under a Class 100 laminar flow hood. The sample solutions were then analyzed in the SSMS (multi-element) or thermal source mass spectrometer (for Pb only).

The first set of bottles were re-filled with a (1+1) mixture of pure HNO_3 and pure water and the entire cleaning, sampling, spiking, analysis sequence was repeated as before. Finally, the bottles were filled with high purity 0.5 percent HNO_3, sampled after one week and again after two months, and then the aliquots were spiked with ^{206}Pb and subsequently analyzed.

NAA Studies. Specimens of 0.2-2 grams (4-12 cm^2) were taken with a steel paper cutter from new 0.5-1 l plastic bottles (CPE, LPE, PP, PMP, PC, PVC, and FEP), sheet (TFE and PFA), bottle caps (ETFE), boxes (PS) or tape (TPT). Precleaning consisted of a light rinse or wipe with ethanol, and then with distilled water. Contamination from the steel paper cutter was eliminated after irradiation.

All irradiations were performed at a thermal neutron flux of 1.3×10^{13} n $cm^{-2}sec^{-1}$ and a gamma flux of 2×10^7 rad/hr (8). Other positions in the reactor offer higher neutron flux, but at the expense of a much higher gamma flux per neutron. Gamma counting was performed on two large-volume Ge(Li) detectors. Peaks used for quantitation contained greater than 50 net counts and better than 25 percent (relative standard deviation) precision from counting statistics. Signals from many elements were obscured by other dominant activities, notably Na, Br, and Cl.

NAA Experiment 1: The plastics were each irradiated for two minutes. After irradiation the edges were trimmed to remove edge contamination and the samples were counted. Then, the samples were cleaned in (1+1) HCl by heating at 100 °C for two hours, rinsed with high purity water, dried, and irradiated and counted again. After a similar two-hour leach in hot (1+1) HNO_3 the plastics were analyzed a third time.

NAA Experiment 2: Fresh samples from the same plastics were used in measurements of long-lived activation products. After irradiations (4-6 hrs) and decay of short-lived activities the edges were trimmed and the plastics were counted. Then the samples were leached in (1+1) HCl as before, rinsed, and leached in hot (1+1) HNO_3. The leach solutions and cleaned plastics were then counted.

Results and Discussion

Gravimetric Studies. The results obtained for the materials studied are given in Table 1. In addition, data previously obtained by Moody, et al, (9) indicated an annual rate of water loss of ~0.05 percent from one liter Teflon bottles. PVC, PMP, and PC containers may be excluded from consideration as container materials due to their high permeability to water. Both Teflon FEP and PP containers have a rate of loss of less than 0.1 percent per year. CPE containers lost slightly more than 0.1 percent. Such losses may be minimized or largely eliminated by sealing the bottle in a bag made of a commercially available vapor barrier material i.e. polyethylene coated aluminized mylar. None of the observed loss rates should be construed as permeability data since the closure may have been responsible for a significant portion of the total water loss.

All containers used were bought from commercial sources and are believed to be similar to those most often used in the laboratory. Three of the containers (PP, FEP, and CPE) have water loss rates which are compatible with the long term storage of samples while the others (PVC, PMP, and PC) have significant rates of water loss. The results obtained for CPE are similar to those observed by Curtis, et al, (10). Humidity conditions during the study averaged about 40 percent R.H. The practice of using a desiccator partially filled with water to act as a humidity chamber to slow transpiration losses is recommended although one must be careful that high humidity conditions do not cause a weight gain (absorption of moisture).

IDMS Studies. The results obtained for the leaching of lead from FEP, LPE, CPE, and PC containers are listed in Table 2. Lead was chosen as a model element for this study, partly because of the high accuracy obtainable by thermal source isotope dilution mass spectrometry. The sequence of using HCl first and then HNO_3 conforms to long standing practice in our laboratories (7). Most of the cleaning of these containers is accomplished by only one week of soaking in HCl. The additional week of soaking in (1+1) HNO_3 removed additional lead only from FEP and PC.

Patterson (2) has suggested that 0.5 percent HNO_3 is more efficient for cleaning Teflon containers of Pb contamination than is (1+1) HNO_3. This was tried after leaching with (1+1) HNO_3 no further leaching of lead was detected except from the PC container. After one month of leaching with 0.5 percent HNO_3, no further lead was leached even from the PC container. The greater quantity of various elements leached from FEP may be partially explained by the higher temperature (80° vs room temperature) used to clean the Teflon FEP container. One advantage of Teflon is that it is chemically inert. The extent of chemical attack on other materials can be quite severe even at temperatures only slightly above room temperature.

Data obtained by SSMS is compiled in Tables 3 and 4 for impurities leached by (1+1) HCl and (1+1) HNO_3 respectively. Again, the Teflon FEP bottles were heated to 80 °C while the others were leached at room temperature. Generally, the (1+1) HCl leached more than the (1+1) HNO_3 except for the FEP containers. A single element, Ca, is responsible for most of the difference between the (1+1) HCl and (1+1) HNO_3 in leaching Teflon FEP.

Values which are below 2 ng/cm^2 or which are prefixed by ≤ are upper limit numbers. That is, the concentration is below the optimum concentration range for the amount of spike isotope used or the concentration is near the blank or detection limit.

NAA Studies. The amount of impurities found in the uncleaned plastics as determined in NAA experiments 1 and 2 are summarized in Table 5. A comparison of the impurities found in CPE in this study shows agreement with that found by other workers (4,12-16) to approximately an order of magnitude. It is interesting to note that there has been no clear change in trace elemental impurities in over 25 years of manufacture (13). The ten materials studied show striking differences in trace element composition. The purest materials are TFE and CPE, in agreement with the experience of numerous workers. Polystyrene, cut from a box used to package NBS Standard Reference Materials, was also very clean. Many materials showed easily detectable amounts of a few elements. Linear polyethylene contained large amounts (>10 ppm) of Na, Al, Ca, Cl, and Zn; polypropylene-Al, Cl, and Ti; polymethylpentene-Zn; polycarbonate-Cl and Br; polyvinylchloride-Na and Sn; Teflon FEP-K (and 1 ppm of W); Teflon PFA-Cl; Teflon pipe tape-Al, Zn, and large amounts of Ti; and ETFE-large amounts of Cl. Some of these elements are residues of polymerization catalysts (17).

The results of the acid leaching experiments are presented in Table 6. They are compiled from the differences in trace element composition of the plastic after leaching in the first and second NAA experiment, and from direct measurement of leached tracer in the second. The two experiments are generally concordant in the conclusion that less than half of most trace elements are leachable, and quantitatively concordant in the results for sodium, the only element common to the two NAA experiments. This point is important because the long irradiation in the second experiment makes those measurements an imperfect model of a reagent or analyte stored in a bottle.

Those atoms of trace elements leached and detected in the leachate in the second experiment are precisely those not representative of undisturbed trace elements in an undisturbed matrix. The radioactive atoms underwent recoil on absorption of a neutron and thus may have been more labile than their inactive neighbors. In addition, the matrix suffered radiation damage from some 10^8 rads of gamma radiation, with the result that all materials were visibly browned and their physical properties were greatly changed. The halocarbons were damaged additionally by the absorption of nuclear recoil energy and beta radiation induced in major constituents of the matrix. Teflon TFE and especially pipe tape were perceptibly embrittled, and PVC became black after the long irradiation.

With the exception of Na in pipe tape, (perhaps a special case because of its high surface/volume ratio), and to a lesser extent Na in CPE, the trace elements studied are in general not leached from the polymer matrix even with the rather severe acid treatment used here (note that the rule of thumb of a doubling of reaction rate for every 10 °C rise in temperature makes our conditions correspond to a few weeks' immersion at room temperature). It may be inferred that generally the bulk of trace elements present are distributed throughout the matrix, and not merely on the surface.

The hydrochloric acid leach in experiment 1 increased the chloride concentration in all the plastics, but not grossly so. The added chloride contamination corresponds to a 10^{-4} cm thickness of 1:1 HCl in the surface of polypropylene, and less in the other materials. Neither this chloride nor other elements were entirely removed by the subsequent HNO_3 leach. This inward diffusion of HCl is in accord with the water loss measurements, both experiments leading to a diffusion coefficient for aqueous solutions in polyolefin of $D=10^{-12}cm^2/sec$. If the removal of trace contaminants depends on diffusion of water as a rate-limiting step, then the inertness of trace elements within the matrix is explained. With this value of D, the mean square diffusion length in one year $\bar{x} = \sqrt{2\ Dt} = 0.1$ mm.

The observation that a second leach removes much less contamination than the first indicates that only surface contamination is easily removed. The cleanest plastic materials available must be cleaned further for the lowest blanks. Many methods have been suggested and several have been shown to be sufficient for the purpose at hand (1,2,7). Since continued cleaning with an array of purest solvents would continue to clean any container (1), the ideal cleaning method will be forever out of reach.

Conclusions

Several commercially available plastic containers have been examined for rate of transpiration of water. Those which have been found to be suitable include Teflon FEP, and PP. With a suitable moisture barrier, CPE containers can also be held to water losses of less than 0.1 percent per year. It is recognized that there can be considerable bottle-to-bottle variation in wall thickness and other parameters of construction. Results of the trace element studies are especially suspect in this regard since even one occlusion in the container wall can considerably affect the trace element levels.

The containers used for these studies were carefully selected to be free from visible occlusions and were average in wall thickness, weight, and other characteristics. While the trace element concentrations found by NAA and IDMS cannot be said to be statistically representative of all bottles of these types, the large differences observed between various plastics are probably valid. In summary, the best containers are believed to be those constructed of CPE and the various Teflons. Differences were observed between NAA and IDMS results for trace elements leached from the plastics. At least some of these differences may be procedural since all the NAA samples were heated whereas only the FEP samples were heated for the IDMS study.

Another possible difference between the two results may be in the way the samples were leached. For IDMS work the leaching was carried out within intact bottles and the results are believed to be representative of the contamination to which a sample would be exposed in an uncleaned bottle. In contrast, the NAA studies examined the trace elements leached from both sides of a small sample of plastic immersed in acid. Since at least some containers are known to be blow molded, the higher results obtained by NAA may simply reflect a difference in contamination levels between inside and outside walls, the NAA data probably including contamination from the mold.

With the exception of Teflon FEP, (1+1) HCl has been found to be the better cleaning agent. However, HCl and HNO_3 appear to leach various elements with different efficiencies, thus, the use of both acids one after the other is recommended. It should not be surprising that in sequential cleaning studies (for Pb by IDMS, various elements by NAA), most of the cleaning is accomplished in a short period of time. From the present work, that of Karin et al., (16), and other work in this laboratory (3,7) a procedure may be suggested that is optimum for most trace work: First, fill the containers with reagent grade (1+1) HCl and allow them to stand for one week. Empty, rinse with distilled water, and re-fill the containers with (1+1) HNO_3 and allow to stand for another week. Finally, empty, rinse, fill with the purest available distilled water, and allow to stand until needed. Preferably, the distilled water should be changed periodically to assure continued cleaning. Teflon bottles should be heated through the first two acid leaches.

Finally, it should be recognized that despite all efforts to clean these bottles, the data would seem to indicate that only the surfaces of the container walls have been cleaned. While very low blank levels may be achieved in a relatively short period of time (several weeks), there is insufficient data to suggest what levels of contamination might occur over a very long period of storage. One possible solution to this problem, depending upon the sample involved, would be to freeze the sample for long term storage.

Then there is the matter of the cost of the containers. Currently, there is greater than one order of magnitude difference in cost between FEP and CPE. Teflon is the preferred container but its cost often mitigates against its use. Other plastic materials are available which might be suitable for some applications. However, we have deliberately chosen to examine only those materials which are commercially available in suitable containers. The analyst should keep abreast of changes in materials and methods of fabrication which would have an influence on our results.

Acknowledgement

The authors wish to acknowledge the contributions of T. J. Murphy, P. J. Paulsen and J. W. Gramlich for the IDMS analysis of solutions stored in plastic containers.

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Table 1. Annual Rate of Loss of Water from Container Materials.

Container	After 17 Days	After 66 Days
CPE	0.116%	0.109%
PP	0.034%	0.049%
PVC	0.429%	0.601%
PMP	0.988%	1.018%
PC	1.65%	2.00%

Table 2. Lead Leached from Containers (ng/cm^2).

Bottle	(1+1) HCl	(1+1) HNO_3	0.5% HNO_3	0.5% HNO_3
	1 week	1 week	1 week	2 months
FEP	0.41	0.014	---	---
LPE	0.20	---	---	---
CPE	0.18	---	---	---
PC	0.36	0.023	0.023	---

--- no significant amount over blank level

Table 3. Impurities Leached from Plastic Containers by (1+1) HCl (ng/cm^2).

Elements	Teflon FEP	LPE	CPE	PC
Pb	2	0.6	18	10
Tl	$\leq$1	$\leq$0.6	3	0.7
Ba	2	1	0.3	3
Te	2	---	0.7	---
Sn	1	$\leq$1	$\leq$0.8	13
Cd	0.6	0.2	0.2	$\leq$8
Ag	$\leq$6	---	---	---
Sr	$\leq$1	0.2	0.2	0.3
Se	0.8	0.4	$\leq$0.3	$\leq$0.5
Zn	4	9	1.0	---
Cu	6	1	0.7	$\leq$6
Ni	0.8	0.8	0.3	0.3
Fe	16	1	1.0	$\leq$49
Cr	4	0.8	0.3	$\leq$5
Ca	2	60	0.8	$\leq$16
K	1.6	1	0.7	$\leq$5
Mg	1.0	0.4	0.7	0.8
Al	4	4	10	3
Na	2	6	42	8
	Σ58	Σ89	Σ81	Σ130

Table 4. Impurities Leached from Plastic Containers by (1+1) HNO_3 (ng/cm^2).

Elements	Teflon FEP	LPE	CPE	PC
Pb	2	2	0.7	0.3
Tl	$\leq$1	$\leq$1	1	$\leq$0.8
Ba	4	$\leq$0.2	2	0.3
Te	0.6	0.2	$\leq$0.5	0.3
Sn	1	1	$\leq$0.8	0.2
Cd	0.4	0.2	0.2	0.3
Ag	$\leq$8	0.2	---	---
Sr	0.2	1	0.2	$\leq$0.2
Se	0.2	0.4	3	0.5
Zn	4	8	2	0.8
Cu	2	0.4	2	0.8
Ni	2	1.6	0.5	0.7
Fe	20	3	3	3
Cr	0.8	0.2	0.8	0.3
Ca	80	0.6	10	3
K	2	2	2	2
Mg	8	0.6	0.7	2
Al	6	1	1	5
Na	6	10	8	3
	Σ148	Σ50	Σ38	Σ23

Table 5. Concentrations of Trace Elements in Plastics (μg/g).

	CPE	LPE	PP	PMP	PS	PC	PVC	TFE	FEP	PFA	TPT	ETFE
Na	1.3	15	4.8	0.20	2.2	2.7	20	0.16	0.40	0.1	2.3	0.6
Al	0.5	30	55	6.2	0.5	3.0		0.23	0.20		29	
Cl	7	30	180			50	major		0.8	50	7	1000
K	5[a]	0.6[a]		0.2[a]					93		3	1.1
Ca		800										
Ti		5	60	5	1						2000	
Mn		0.02	0.02	0.01	0.02				0.06	0.02	0.02	
Co			0.04			0.006[a]				0.09		
Zn		520		33							14	
Br	0.02[a]	0.8	0.005[a]	0.002[a]	0.001[a]	29	0.006[a]	0.002[a]		0.16		0.24
Sn							2400					
Sb	0.005	0.2	0.6									
La					0.0003			0.0006				0.001[a]
W									0.7			
Au			0.0001[a]	0.0006	0.00004[a]	0.00003[a]		0.0003			0.0002[a]	0.0004[a]

[a] Certain elements were detected only in leach liquids. ^{24}Na or ^{82}Br usually predominated in the plastics even after cleaning, consequently trace elements not removed from the matrix were not determinable; starred concentrations are lower limits. Blank spaces imply that the element was not detected.

Table 6. Sequential Leaching of Trace Elements from Plastics (ng/cm^2)[a]

	CPE	LPE	PP	PMP	PS	PC	PVC	TFE	FEP	PFA	TPT	ETFE
Na	200+0.4	70+1	100+7	20+0.4	20+8	20+1	30+10	80+	60+	20+	50+4	300+1
Al	50+											
K	1000+	100+		80+					90+			400+
Co						+2						
Zn		50+30									20+	
Br	3+	2+0.5	1+	1+	0.3+0.1	7+20	2+	10+				
Sb	1+	3+										
La					0.1+			0.4+				0.7+
W									4+0.2			
Au			0.03+0.01		0.01+	0.01+		0.3+			0.01+	0.3+0.01

[a] The notation "N+M" implies that N ng of the element in question was leached from 1 cm^2 of the plastic by hot (1+1) HCl in one hour, and M ng subsequently leached by (1+1) HNO_3. Blank spaces imply that the element was not detected.

APPENDIX B

EVALUATION BY ACTIVATION ANALYSIS OF ELEMENTAL RETENTION IN BIOLOGICAL SAMPLES AFTER LOW TEMPERATURE ASHING

by

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INTRODUCTION

Low temperature ashing (LTA) is the low temperature (~100 °C) decomposition of organic or biological samples with atomic oxygen produced by an electrodeless radio-frequency (RF) discharge. There are a number of advantages in using LTA as the first step in an analytical method whether by nuclear activation or otherwise:

1. Since the oxidation temperature is low, volatility losses are minimized during the destruction of large quantities of organic material.

2. There are no electrodes to contaminate the sample.

3. A sample can be more readily dissolved after LTA than by alternative methods. Wet ashing techniques with hot oxidizing acids or strong alkalis are potentially hazardous, sometimes incomplete, and usually susceptible to contamination because of the large amounts of reagents required.

4. High temperature ashing in a muffle furnace introduces the possibility of contamination from containers or the furnace walls as well as loss of some elements by volatilization. The convenience of the LTA method can be demonstrated by reference to a publication concerning the spectrophotometric determination of boron extracted from ashed animal tissues (1). Four grams of freeze-dried tissue were ashed 40-50 hours at 100-150 watt forward RF power. After the LTA treatment, the sample could be dissolved in 2 mL of 1N HCl.

5. In the X-ray fluorescence method, which is strongly matrix dependent, LTA reduces the sample to a silicate-carbonate-oxide form, which minimizes errors due to that problem.

6. In spark-source mass and emission spectrometry the ashed residue, when mixed with powdered graphite, gives a uniform consistency which also reduces matrix error.

With specific respect to activation analysis, one can identify several advantages of beginning the analysis of a biological sample with freeze-drying followed by LTA.

1. A very high neutron flux can badly char a biological or organic sample leavig it with an undesirable consistency. That the LTA method is very efficient for removing organic carbon is demonstrated by an American Society for Testing and Materials procedure (2) which specifies the technique

in removal and hence determination of organic carbon as opposed to carbonate in sediment samples.

2. LTA results in a substantial reduction in the volume of the sample. This can yield a corresponding reduction in electron accelerator or neutron generator irradiation time in photon or 14-MeV neutron activation analysis.

3. Since lengthy wet digestions are eliminated, a sample after irradiation can be dissolved more quickly and hence short-lived nuclides can be more quickly separated from the sample for counting.

4. In the instrumental photon activation analysis of samples containing large amounts of carbon, it is usually necessary to delay counting for a few hours after irradiation to allow the ^{11}C produced by the reaction $^{12}C(\gamma, n)^{11}C$ to decay. Some shorter-lived nuclides are therefore not detected. Many of them could be detected by removal of the carbon prior to irradiating.

The possibility exists in the LTA method of the loss of elements of interest during the ashing process. Studies to evaluate elemental retention during ashing have been conducted with radioactive tracers (3). This is not an entirely satisfactory procedure since a number of elements may not be present in a biological sample in the same chemical form as was used in the tracer studies. This paper describes a study, using activation analysis, of loss or contamination of trace elements during LTA. It is hoped that this will be useful not only in analytical chemistry for the reasons listed above, but also to evaluate LTA as a potential processing method for the retention of biological samples by the National Environmental Sample Bank over several decades of time for purposes of baseline studies to evaluate increases or decreases in pollution.

EXPERIMENTAL

A commercial model LTA apparatus capable of 100 watts forward power was used in these experiments. A schematic of the apparatus is shown in figure 1. The sample was placed in a borosilicate glass ashing chamber which was briefly rotated by hand to induce the organic sample to spread as evenly as possible on the inside surface. The cold trap was filled with crushed dry ice (liquid nitrogen would condense explosive ozone). The system was pumped down to 1.3-2.6 Pa (10-20 millitorr). The oxygen flow rate was adjusted so as to maintain a pressure of 13-40 Pa (100-300 millitorr). Forward power was usually set to the maximum of 100 watts. Tuning to optimum was accomplished by observing the maximum intensity in discharge glow in the tube.

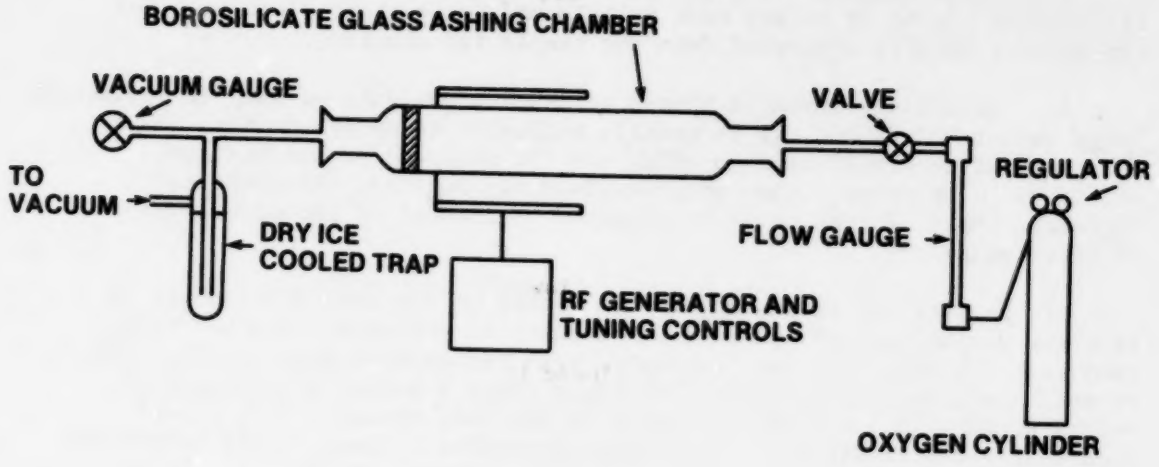

Figure 1. Block diagram of LTA apparatus.

Preliminary experiments involving addition of tracers to biological materials and determining retention of each isotope after ashing verified previously published data (3) and served to establish and perfect the basic ashing technique. After these experiments, it was decided to study three NBS Standard Reference Materials in detail; 1571-Orchard Leaves, 1577-Bovine Liver, and 1632-Trace Elements in Coal.

Samples of about one gram were ashed for a period of several hours. It was impossible to remove all of the ashed material from the tube, thus it was necessary to determine the exact amount of material removed. This was first attempted by weighing of the tube which proved difficult, since the only balance capable of accommodating the 200 g in weight, 30 cm in length ashing tube was accurate to only ± 2 mg. A second and more successful technique involved the use of a nonvolatile radioactive tracer that wouldn't

interfere with later activation analysis. The uniform distribution of the tracer throughout the bulk sample was demonstrated by taking several small samples from the bottle and measuring their specific activities. Subsequently, it was found possible to use the activation product of an element demonstrated to be nonvolatile as an internal standard for this purpose.

The relative amounts of the elements in ashed and unashed samples were determined by nondestructive neutron activation analysis with the NBS 10 megawatt nuclear reactor and photon activation analysis with the NBS electron linear accelerator.

For the reactor irradiation a paired experiment was carried out using approximately one gram of unashed material compared to the ashed residue of one gram of material. Three series of neutron irradiations were conducted:

1. Fifteen-second irradiation followed by three countings at few minute intervals,

2. Twenty-minute irradiations followed by 2-3 countings over a period of one hour to a few days,

3. A four-hour irradiation followed by 2-3 countings over a period of several days to a few weeks after irradiation (depending on Na-24 levels).

The two shorter irradiations were conducted at a flux of $1.3 \times 10^{13} n \cdot cm^{-2} \text{-} sec^{-1}$, and the longer irradiation at a flux of $5.6 \times 10^{13} n \cdot cm^{-2} \text{-} sec^{-1}$.

Two series of photon irradiations were conducted with approximately three grams of unashed material and the ashed residue of three grams of material with an electron energy of 35 MeV and a beam current of about 50 μA.

1. Thirty to sixty minute irradiation followed by 2-3 countings over a period of two hours to a few days after irradiation,

2. Two to six hour irradiation followed by 2-4 countings over a period of two days to a few weeks after irradiation.

Irradiated samples were counted with a 75 cc Ge(Li) detector and a 4096 channel analyzer using one-half of the memory. Molybdenum, iodine, and zinc by photon activation analysis were also determined with a low energy photon detector (LEPD). Photopeak counting rates were corrected for times of irradiation and decay and ratio of weights of unashed material and ashed residue corrected for weight loss.

RESULTS AND DISCUSSION

The experimental study of the observation of ashing losses was carried out in three phases. The first consisted of spiking samples of interest with radioactive tracers of known chemical states. This phase was simple to conduct as the isotopes could be so added to avoid interference in radioactive counting. This first phase severed to assist in development of handling techniques for subsequent work. The results with radioactive tracers showed the retention of zinc, cadmium, arsenic, palladium, rhenium, antimony, iron, platinum, iridium, gold, and silver. A previous paper (3) had reported the loss of gold and silver but this phenomenon was not confirmed here. Mercury and osmium were lost after several hours of ashing in every case.

The second phase consisted of irradiating portions of the three samples, counting them, then ashing and recounting. These experiments added to the retention list the following elements: sodium, potassium, rare earths, and selenium.

The third phase, which was the major part of this study, involved the simultaneous activation, either by thermal neutrons or high-energy photons, of an untreated portion of the samples and an ashed portion. This study thus established loss data on elements in the chemical form they would actually be found in three representative matrices. Table 1 gives typical weight losses and carbon losses as measured by the photonuclear reaction $^{11}C(\gamma,\alpha n)^{7}Be$ for an ashing period of approximately 12 hours and power of approximately 70 watts. In addition to carbon, the loss of chlorine, bromine, and iodine was observed in all three matrices as well as the expected loss of mercury. Table 2 gives experimental recoveries of retained elements.

TABLE 1. TYPICAL WEIGHT AND CARBON LOSSES FOR ASHING PERIODS OF 15 HOURS AND RF POWER OF 70 WATTS

	% Weight Lost	% Carbon Lost
Coal	80	75
Beef Liver	90	98
Orchard Leaves	75	80

Figure 2 shows, in the formate of the periodic chart, the elements detected and not lost during ashing in all experiments. The symbols in the individual square of each element, OL, C, and BL stand for orchard leaves, coal, and beef liver, respectively, in which the element was determined. In addition, some elements reported retained in the literature determined by neutron activation analysis (4), atomic absorption spectrometry, (5) or spectrophotometry (1) are included. The numbers refer to references.

H																HE
LI	BE											B 1	C	N	O	F	NE
NA C OL BL	MG C OL BL											AL C OL BL	SI	P	S	CL	AR
K C OL	CA C OL BL	SC C OL BL	TI C OL	V C	CR C OL BL	MN C OL BL	FE C OL BL	CO C OL BL	NI C OL	CU 5	ZN C OL BL	GA C	GE	AS C OL	SE C OL BL	BR	KR
RB C OL BL	SR C OL	Y	ZR C OL	NB	MO OL BL	TC	RU	RH	PD	AG 4	CD 5	IN	SN	SB C OL BL	TE	I	XE
CS C OL BL	BA C OL BL	LA C OL BL	HF C OL	TA C	W C	RE	OS	IR	PT	AU	HG	TL 5	PB C OL	BI	PO	AT	RN
FR	RA	AC															

CE C OL BL	PR	ND	PM	SM	EU C OL BL	GD	TB	DY	HO	ER	TM	YB	LU
TH C OL BL	PA	U	NP	PU	AM	CM	BK	CF	ES	FM	MD	NO	LW

1. J.W. Mair, Jr., and H.G. Day, Anal. Chem. 44 (1972) 2015. Matrix was animal tissue.
4. D.Behne and P.A. Matamba, Z. Anal. Chem. 274 (1975) 195. Matrix was blood serum.
5. B.B. Stafford, Proceedings 2nd Conf. Trace Subs. in Environmental Health (1968) Univ. of Mo. Matrix was atmospheric particulate.

Figure 2. Elements retained during LTA.

Although some of the results have been previously reported, we include data here on many additional elements, including those particularly susceptible to loss such as arsenic, selenium, and chromium, since they are known to form compounds which are volatile at the temperatures of LTA.

TABLE 2. PERCENT OF ELEMENT RETAINED (EXPERIMENTAL)

	Coal	Orchard Leaves	Beef Liver
Sodium	98	100	104
Magnesium	98	101	95
Aluminum	105	-	112
Potassium	101	106	-
Calcium	99	101	99
Scandium	100	101	-
Titanium	105	99	-
Vanadium	98	-	-
Chromium	99	106	94
Manganese	102	102	105
Iron	96	102	99
Cobalt	108	95	106
Nickel	99	99	-
Zinc	107	102	100
Gallium	91	-	-
Arsenic	110	92	-
Selenium	99	105	-
Rubidium	98	98	92
Strontium	98	106	-
Zirconium	104	108	-
Molybdenum	93	94	-
Antimony	104	108	-
Cesium	102	104	-
Barium	105	109	-
Lanthanum	107	95	95
Cerium	99	98	100
Europium	98	-	94
Hafnium	96	98	-
Tantalum	109	-	-
Tungsten	97	-	-
Lead	106	97	-
Thorium	103	92	-

It is also significant to note that, equal in importance to loss of trace elements, there was no pick-up of contamination during the ashing process as would be evidenced by an increase in the amount of the element after ashing. This is in spite of the fact that only very ordinary precautions were taken to guard against contamination during cleaning of the borosilicate glass ashing tube with ordinary laboratory detergent followed by rinsing with water, hot dilute hydrochloric acid and finally deionized water and drying in an oven at 100 °C.

An application of immediate interest is those elements which upon thermal neutron or photon activation yield radioisotopes with half-lives in the range of 2-30 minutes and are not detectable in the typical biological sample without separation. Since after ashing, biological samples, especiall animal tissue, are rapidly dissolved in dilute mineral acid, one may consider radiochemical separations in the determination of magnesium, aluminum, titanium, and vanadium in the case of thermal neutron activation and potassium and iron in photon activation.

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APPENDIX C

DETERMINATION OF CHROMIUM IN BIOLOGICAL MATRICES BY NEUTRON ACTIVATION: APPLICATION TO STANDARD REFERENCE MATERIALS

by

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ABSTRACT

Chromium is recognized to be an essential trace element in several biological systems. It exists in many biological materials in a variety of chemical forms and very low concentration levels which cause problems for many analytical techniques. Both instrumental and destructive neutron activation analysis were used to determine the chromium concentrations in Orchard Leaves, SRM 1571, Brewers Yeast, SRM 1569, and Bovine Liver, SRM 1577. Some of the problems inherent with determining chromium in certain biological matrices and the data obtained here at the National Bureau of Standards using this technique are discussed.

INTRODUCTION

Chromium has been recognized as an essential trace element (1) in human nutrition for several years. At high concentrations or in different chemical states it can also cause deleterious or toxic effects. There is a need for reliable methods of analysis for chromium at trace levels in a variety of matrices, especially biological matrices. A laboratory intercomparison on environmental materials (2) pointed up the sad state of chromium determinations as performed by most of the analytical community. Also, the author has observed a wide range of results reported for chromium in the National Bureau of Standards (NBS) Bovine Liver Standard Reference Material (SRM 1577). As a result of these observations and communication with several analytical laboratories involved in the determination of chromium in biological matrices, persons at NBS became convinced that more biological materials needed to be analyzed and certified for chromium content, in addition to other elements.

Neutron activation analysis, both instrumental (INAA) and destructive (DNAA), has been used to determine the chromium concentration in a variety of matrices. The results obtained for chromium in three biological Standard Reference Materials (SRM's)--Orchard Leaves, Bovine Liver, and Brewers Yeast--are described in this paper. The chromium content has been certified by NBS in two of these SRM's, Orchard Leaves and Brewers Yeast.

EXPERIMENTAL

Samples Analyzed

Two of the biological materials analyzed were NBS Standard Reference Materials Orchard Leaves (SRM 1571) and Bovine Liver (SRM 1577). The third material, Brewers Yeast, was furnished to NBS by the Nutrition Institute, U.S. Department of Agriculture, Beltsville, Maryland, for preparation, certification, and issuance as a Standard Reference Material. This material is certified for total chromium concentration only and is being prepared for sale by the NBS Office of Standard Reference Materials as SRM 1569.

Preparation of Standards and Carrier

Two standards were used in this work. One standard was prepared by dissolving a weighed amount of chromium metal (99.99% pure) in high purity HCl and diluting to a specific volume with high purity H_2O to obtain the desired Cr concentration. The second standard was prepared by dissolving

a weighed amount of NBS-SRM 136C ($K_2Cr_2O_7$) in high purity H_2O and diluting to volume to obtain the desired Cr concentration. Chromium carrier solutions were prepared from analytical reagent grade $CrCl_3$ and $K_2Cr_2O_7$ and normally contained 3 mg Cr/mL.

All reagents used in the analysis were of analytical grade, unless stated otherwise.

Irradiation Conditions

Chromium was determined in the three biological materials using both INAA and DNAA. The samples (200-350 mg) along with Cr standards were encapsulated in clean quartz vials and irradiated for periods of one to six hours in the NBS Reactor at a thermal neutron flux of $1x10^{13}$n $cm^{-2} \cdot sec^{-1}$. The samples were allowed to decay 3-6 weeks for INAA work and ∿36 hours for DNAA work to reduce the matrix activity before processing.

Procedure for INAA

Following a three-week decay the sample vials were washed clean of exterior contamination with 1:1 HNO_3 and H_2O, frozen in liquid nitrogen opened, and the material was transferred to clean polyethylene counting vials. The amount of sample transferred was determined by weight. The samples and standards were counted on a 75 cm^3 Ge(Li) detector coupled to a 4096 channel pulse height analyzer for measurement of ^{51}Cr produced by the $^{50}Cr(n,\gamma)\ ^{51}Cr$ reaction. The concentration of chromium was determined by the direct-comparator method.

Several brewers yeast samples and standards were preweighed into clean quartz vials, sealed, and irradiated as already described. After allowing these samples to decay for ∿6 weeks, the vials were washed clean of exterior contamination and counted (material still contained in vial) as already described.

Procedure for DNAA

Following a 36-hour decay, the sample and standard vials were cleaned, frozen in liquid N_2, opened, and transferred (by weight) to a 50 mL Erlenmeyer flask designed for dissolving materials in a closed system (3) and trapping volatile material in an external solution. Chromium carried (∿5 mg of both Cr III and Cr VI) and 5-10 mL of concentrated $HClO_4$-HNO_3 mixture (1:3) were added to the flask. The flask was heated on a Pyrex top hot plate until all the sample had dissolved and all the vapor fumes visibly trapped in a 1:1 HNO_3 solution. The flask was removed from the heat and 5 mL of hot $Ce(SO_4)_2$-3M H_2SO_4 solution (10% w/v) was added; the flask was heated an additional 5-10 minutes to assure oxidation of and maintain the chromium as Cr VI. After cooling, the sample solution in the flask was transferred to a 50-mL extraction tube with 15 mL of 1.5 M HCl. The sample was then extracted with 10 mL of 1 percent (w/v) tribenzylamine-chloroform solution. A 5-mL aliquot was taken from the organic phase and counted on a 25 cc Ge(Li) detector coupled to a HP 4096 computer-analyzer. The trap solution

from the dissolution step was transferred to a suitable counting vessel and counted on this system also. The 320 keV gamma ray peak of ^{51}Cr was integrated and the chromium concentration was determined by the direct-comparator method using the two chromium standards subjected to the same procedure. All of the chromium from these standards remained in the flask. The trap solution was checked for chromium but none was detected. The procedure is the same for open-flask dissolution of samples except a regular Erlenmeyer flask is used, eliminating the trap solution.

Results and Discussion

Recent interlaboratory comparison studies (4,5) of analytical methods and their results for the analysis of chromium in biological and environmental materials showed very large differences in analytical values. During the spring of 1974, a workshop on chromium analysis was held at the University of Missouri in Columbia where these differences were discussed and recommendations made to resolve them. There was consensus among the participants that biological standard reference materials, certified for total chromium content by NBS, would provide a starting point for the analytical community to critically evaluate their methods.

Chromium has been determined and certified in several materials by NBS. However, this manuscript describes the results of chromium analyses in three biological materials--Orchard Leaves, Bovine Liver, Brewers Yeast--using neutron activation analysis (NAA). Studies here in our laboratory and other laboratories (6,7) on some biological materials had suggested possible losses of chromium content during sample manipulation (e.g., dissolution, charring, ashing, etc.). Instrumental neutron activation analysis (INAA) and destructive neutron activation analysis (DNAA) was used to determine the chromium concentration in these biological materials. The use of INAA to determine the total chromium concentration in these biologicals provided an "absolute" check for the values obtained using DNAA and also other techniques which required sample treatment. The results obtained for chromium in the three biologicals mentioned above, using NAA and the NBS Reactor, are given in Tables 1-4.

By employing two dissolution techniques--closed system and open flask--for the irradiated biologicals, the loss of chromium in the dissolution step could be evaluated. The results obtained for chromium in Orchard Leaves (Table 1) were essentially the same using INAA and the two dissolution techniques, indicating no chromium is lost from this material in the dissolution procedures used. The slightly higher INAA result for chromium is expected. A large amount of matrix radioactivity is produced when these biological materials are irradiated. Much of this activity results in long-lived beta radiation creating a bremsstrahlung effect in the gamma spectra when the sample is counted. This effect is very dominant in low energy range of the spectra where the chromium photopeak is located so that large background corrections are necessary. Radiochemical separation of Cr (DNAA) eliminates the background interference. The results obtained from open-flask dissolution (DNAA) of yeast (Table 2) were consistenlty lower ($\sim$25%) than those obtained instrumentally (INAA). The results

obtained from dissolution of brewers yeast in a closed system as described in the DNAA procedure are in good agreement with the INAA results. The same phenomenon existed in the determination of chromium in bovine liver (Table 3).

The results in Tables 1-3 show chromium can be lost during sample dissolution of two biologicals, brewers yeast and bovine liver. However, no loss of chromium was observed in our experimental design for orchard leaves. The chromium loss observed in brewers yeast and bovine liver is probably an organic component, since there was apparently no exchange with the inorganic chromium added to the sample before dissolution. Further studies to characterize this component are underway in this laboratory.

Brewers yeast was preweighed, packaged, irradiated, and counted in the same container in order to establish that chromium was not lost in the irradiation step. The results in Table 4 show the amount of chromium obtained using a polyethylene irradiation container (INAA-P.E.) are in good agreement with those obtained from a quartz irradiation container (INAA-Quartz).

The radiochemical procedure described for chromium (DNAA) provides the analyst with a simple, rapid, and selective technique for chromium determination in a variety of matrices. The procedure is also adaptable for use by other techniques. Results of chromium analysis (from a variety of techniques) reported in the literature in recent years show very large differences in concentration on the same materials. Thus, it is hoped these two biological materials, certified for total chromium concentration, will help those involved in chromium analysis to evaluate their methods and improve chromium results reported in the literature and elsewhere for analytical quality control samples.

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TABLE 1. CONCENTRATION OF CHROMIUM IN ORCHARD LEAVES, SRM 1571, (µg Cr/gram)

Spl #	INAA	DNAA[A]	DNAA[B]
1	2.590	2.514	2.472
2	2.574	2.489	2.447
3	2.563	2.499	2.484
4	2.567	2.495	2.433
5	2.569	2.501	2.463
6	2.582	2.472	2.481
$\bar{X}$	2.574 ± 0.010	2.495 ± 0.014	2.463 ± 0.020

* NBS Certified Value: 2.6 ± 0.2 µg/g

Note: A - Closed system dissolution.

B - Open flask dissolution.

* Based on results of two independent analytical methods--- This work and isotope dilution mass spectrometry.

TABLE 2. CONCENTRATION OF CHROMIUM IN BREWERS YEAST, SRM 1569 (µg Cr/gram)

Spl #	INAA	DNAA[A]	DNAA[B]
1	2.079	2.070	1.583
2	2.075	2.068	1.543
3	2.090	2.062	1.548
4	2.077	2.068	1.588
5	2.067	2.088	1.551
6	2.104	2.090	1.565
$\bar{X}$	2.082 ± 0.013	2.074 ± 0.012	1.558 ± 0.015

NBS Certified value: 2.12 ± 0.05 µg/g --- Based on results of two independent analytical methods--This work and isotope dilution mass spectrometry.

Note: A - Closed system dissolution.

B - Open flask dissolution.

TABLE 3. CONCENTRATION OF CHROMIUM IN BOVINE LIVER SRM 1577 (μg Cr/gram)

Spl #	INAA	DNAA[A]	DNAA[B]
1	0.228	0.212	0.157
2	0.170	0.209	0.166
3	0.229	0.210	0.153
4	0.242	0.207	0.156
5	0.177	0.214	0.166
6	0.213	0.209	0.160
$\overline{X}$	0.210 ± 0.030	0.210 ± 0.002	0.160 ± 0.005

Note: A - Closed system dissolution.

B - Open flask dissolution.

TABLE 4. CONCENTRATION OF CHROMIUM IN BREWERS YEAST, SRM 1569, Using INAA (μg Cr/gram)

Spl #	INAA - P.E.	INAA - Quartz
1	2.079	2.131
2	2.105	2.087
3	2.117	2.137
4	2.081	2.092
5	2.120	2.149
6	2.075	2.120
$\bar{X}$	2.096 ± 0.020	2.119 ± 0.025